Response: Visual number

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While our brief Report [1] did not discuss previous work on texture density [2–7], we do acknowledge that these studies have some relevance to our own. However, we found some of Durgin's conclusions difficult to reconcile with our findings. From results obtained from two groups of subjects instructed to report either apparent *numerosity* or apparent *density* Durgin [6] claimed that "density is more influential [than numerosity] in the perception of high numerosities". He also suggests that numerosity may derive from what he claims is a correlate of it, *kurtosis*. We are unaware that kurtosis is a correlate of number, and in the case of spots uniformly either white or black the concept of kurtosis is inapplicable.

We instructed all our subjects to report apparent *numerosity* (with no mention of density), by judging whether a test comprised more or fewer elements than a probe after adaptation [1]. All subjects saw the adapted field as containing more or fewer dots than the non-adapted field, even at quite high numerosities. We tried *not* to see the number of dots as increased or reduced, but were unable to do so: adaptation always altered the apparent number of dots, even when the adapting elements were barely above threshold, and this was not under attentional control (which readers are invited to verify with the on-line demonstration. All observers found the effects bewildering, wondering where the missing dots had gone to or the extra dots had come from! We therefore found Durgin's claim that his effect could be abolished by a simple change of instructions difficult to accept.

In his correspondence, Durgin [8] pursues the issue of whether our adaptation effects could not better be described in terms of texture density. He reports new experimental results showing that only dots falling within the spatial region where the test is displayed effectively adapt the region. This result is interesting, and provides a potentially useful technique for mapping perceptive fields responsible for numerosity adaptation; but it does not surprise us — given that the technique we used assumes that adaptation effects are spatially selective. In our on-line demonstration, the left field adapts to many dots, the right field to few, and these adapters *selectively* affect the relevant test patches. It is not the total number of dots that causes the adaptation but only those within a particular area.

Our sense of number is intrinsically linked to our sense of space [9] (see [10] for analogous discussions about form and motion). In so far as we are referring to number within a given region, there is a technical sense that this is not absolute numerosity but numerosity density. But is this useful? If we look at a flock of sheep in a field do we see "1.26 sheep per square meter", or "about 40 sheep in a particular region"? Either way, it does not change our major conclusions.

Is the issue perhaps whether number can be reduced to *texture* density? Given that the number of discrete elements placed in a given area determines texture density, it is clear that adaptation can potentially affect both of them. We did our best to disentangle the two effects, by using elements of mixed contrast polarity, thereby balancing luminance in all displays. We also varied texture drastically, replacing single square dots with lines eight times longer, some vertical, some horizontal, thereby changing pixel density, pattern orientation, Fourier spectrum, first, second and higher-order statistics of the texture, average contrast and almost any other conceivable aspect of texture. None of these manipulations had any effect on the adaptation results.

Even Durgin's [7] own studies would seem to support this view: adaptation of dense random dot stimuli had little effect on the perceived density of 'cloudlike' or 'granite-like' texture, compared with the effect on stimuli comprising distinct objects (compare Figures 2 and 6 of [7]). On the other hand, our (unpublished) studies show strong adaptation between patterns of greatly differing spatial frequency, but comprising distinct, countable objects. Other studies [11] have shown that linking pairs of dots to form dumbbells, changing texture very little, changes apparent numerosity by the amount expect if the links caused two dots to appear as one. All this suggests that numerosity may be sensed directly, independently of size, texture and contrast. Durgin's [8] suggestion, that number is extracted indirectly from a texture representation obtained from the *statistical kurtosis* of a scene evaluated over various scales, seems reminiscent of the legendary Australian stockman who when asked to explain his uncanny ability to judge the number of cattle in a herd replied that he counted the legs and divided by four.

Adaptation of numerosity is consistent with recent physiological studies. Nieder [12] has reported neurons both in pre-frontal and parietal cortex that are tuned for numerosity, even when other factors such as density are well controlled. Recently, Roitman *et al.* [13] uncovered another type of neural code for numerosity in area LIP. The firing rates of some LIP neurons vary monotonically with numerosity (after controlling well for size and density), either increasing or decreasing sharply with the number of objects in the neuronal receptive field. As these neurons have limited and clearly circumscribed receptive fields, they respond solely to the local numerosity within a retinotopic area texture density when controlled for, making them ideal candidates for the neural substrate of spatially selective adaptation to number. In a preliminary study, one of us [14] employed the constant density control for texture density that Durgin [8] suggests we should have: it made no difference.

It is timely of Durgin [8] to remind us that texture is subject to adaptation, as was shown for gratings by Blakemore *et al.* [2], and by MacKay [3] and Anstis [4] for a wider variety of textures, including non-discrete texture, like sandpaper. But just because observers are sensitive to texture differences and because numerosity is linked to texture density, at least for stimuli composed of discrete elements, it seems unwise to conclude that differences in numerosity reduce to differences in texture density. This is particularly so given the increasing number of studies revealing that there are neurones that remain tuned for number even when texture and other possible confounds are carefully controlled.

When there are many elements crowding into limited space we think it likely that mechanisms for detecting number and mechanisms

for detecting the crowding of texture are both at play. Disentangling the two will be an interesting challenge. One promising line, as Durgin [8] suggests, will be to look for dissociations between number and texture perception. Manuela Piazza, Stanislas Dehaene and Marco Zorzi (personal communication) have shown that dyscalculic individuals have higher Weber fraction for number discrimination than do controls. It would be interesting to study whether discrimination of texture is also affected in these individuals; and to test adaptation to both attributes.

We suspect that the investigation of numerosity as a visual primitive will open a rich vein of connections between visual perception and mathematical intuition. Mathematicians often 'see' their solutions first and verify them later, as many testified to Hadamard [15].

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Horizontal gene transfer and the evolution of cnidarian stinging cells

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Genes are regularly transmitted vertically, within one lineage, from one generation to the next, but they can also be exchanged between lineages by horizontal gene transfer (HGT). HGTs are frequent in prokaryotes and have been shown to play important roles in unicellular eukaryotes, whereas only a few instances are known in animals [1,2]. Here, we provide evidence that a subunit of bacterial poly-γ-glutamate (PGA) synthase was transferred to an animal ancestor by HGT. We suggest that this gene acquisition had important consequences on the evolution of the stinging cells (nematocytes) that cnidarians (sea anemones, jellyfish, corals etc.) essentially use to capture prey.

We were alerted to the possibility of a significant HGT from bacteria to metazoans by the unusual phylogenetic distribution of the polyanionic polymer PGA, which has been only detected extracellularly in some prokaryotes [3], and intracellularly in the capsule of cnidarian nematocytes [4–6]. By attracting cations within the capsule, PGA is critical for the nematocyte discharge, which involves rapid changes in intracapsular osmotic pressure [4]. In bacteria, three *pgs* genes, *AA*, *B*, *C*, have been identified as essential for PGA synthesis [3]. We could detect clear orthologues of *pgsAA* in all available cnidarian genomes (Supplemental Data). In the *Clytia hemisphaerica* medusa (Hydrozoa), *pgsAA* is expressed in the nematogenic area of the tentacle bulb, in the same territory as the nematogenesis marker *minicollagen3-4a* [7] (Figure 1A,B). In the same region, we detected a high quantity of intracellular glutamate, the monomer for PGA synthesis (Figure 1C) and strong expression for two glutamate high affinity transporters (Figure 1D,E). These results are consistent with an involvement of pgsAA in PGA production in nematoblasts.

To investigate the evolutionary origin of the cnidarian *pgsAA* gene, we searched for homologues of *pgs* genes in all available complete genomes from Bacteria, Archaea and Eukaryota (Supplemental data). The vast majority of eukaryotes lacked any *pgs* genes, with only the *pgsAA* subunit detected in a few species belonging to various distantly related eukaryote taxa (Figure 2). The patchy distribution of these eukaryote *pgsAA* homologues within the *pgsAA* tree suggests that they were acquired through several independent HGT events. Furthermore, the clear polyphyly of the major prokaryotic *pgsAA* groups suggests that this gene is highly mobile. Consistent with this, *PgsAA* was detected in no less than five naturally occurring plasmids, providing a possible explanation for HGT over large taxonomic distances. The presence of introns with stop codons in several of the eukaryote *pgsAA* genes, including all but one cnidarian *pgsAA* sequences, might provide a directionality to the HGT from bacteria to these eukaryotes; it is highly unlikely that a gene with intronic stop codons could be successfully transferred from Eukaryotes to Bacteria, in which transcription and translation are directly coupled. All but one of the cnidarian *pgsAA* sequences were grouped in our analyses in a single clade (Clade 1), together with a sequence from the sponge *Amphimedon queenslandica* (Figure 2), while most remaining eukaryote sequences were grouped with a subset of bacterial and archaeal *pgsAA* sequences (Clade 2). An AU test rejected the grouping of Clade 1 sequences with any other eukaryote sequence, indicating that the HGT event occurred in an exclusive ancestor of sponges and cnidarians (Supplemental data). Assuming the classical phylogenetic position of the sponges as the sister group of all other metazoans, we can conclude that this HGT dates back to the root of metazoans, and that the bilaterian animals have secondarily lost the gene. Additional independent transfers of *pgsAA* gene from Bacteria to eukaryotes are inferred within Clade 2 (Supplemental data). In particular, the sea anemone *Nematostella vectensis* harbours a Clade 2 *pgsAA* gene branching with sequences